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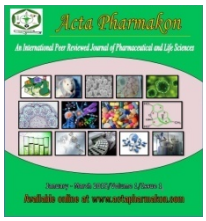

Research Article

Comparative Pharmacognostic Evaluation and HPTLC Analysis in different parts of *T. chebula* Retz.

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ABSTRACT

T. chebula has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. The observed health benefits may be credited to the presence of the various phytochemicals like polyphenols, terpenes, anthocyanins, flavonoids, alkaloids and glycosides. Ethanolic extracts from *T. chebula* fruit, bark & stem are screened for antioxidant activity using, DPPH free radical scavenging activity. Methanolic extract has been studied through HPTLC. *In-vitro* antioxidant study of leaves; fruits and bark of *T. chebula* was performed using ethanolic extract. In this study fruit extract showed the least IC_{50} value of 0.13 mg/ml followed by bark and leaves. Standard quercetin showed IC_{50} value of 0.0072 mg/ml. Quantification of lupeol in the samples of *T. chebula* fruit, bark & leaves has been performed and was found to be 0.042%, 0.024% and 0.034% respectively. Lupeol present in this species has been studied through HPTLC. The presence of lupeol has not yet been compared in different parts in this species which may be utilized for the proper standardization of the drug. Thus, the effective source of *T. chebula* could be employed in all medicinal preparations to combat myriad diseases associated with oxidative stress and related disorders.

1. INTRODUCTION

Traditional healing system around the world that utilizes herbal remedies are an important resource for the discovery of modern drugs. [1] The fruit of the tree possesses diverse health benefits and has been used as traditional medicine for household remedy against various human ailments since antiquity.[2-4] *T. chebula* has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. The observed health benefits may be credited to the presence of the various phytochemicals like polyphenols, terpenes, anthocyanins, flavonoids, alkaloids and glycosides.

The fruit is mild laxative, stomachic, tonic, alterative, antispasmodic. It is useful in opthalmia, hemorrhoids, dental caries, bleeding gums, ulcered oral cavity. Its paste with water is found to be anti-inflammatory, analgesic and having purifying and healing capacity for wounds. Its decoction is used as gargle in oral ulcers, sore throat. Its powder is a good astringent dentifrice in loose gums, bleeding and ulceration in gums. It is good to increase appetite, digestive aid, liver stimulant, stomachic, gastrointestinal prokinetic agent, and mild laxative. The powder of *T. chebula* fruits has been used in chronic diarrhea. It is used in nervous weakness, nervous irritability. It promotes the receiving power of five senses. It is adjuvant in hemorrhages due to its astringent nature and good for chronic cough, chorizo, sore throat as well as asthma. Also it is useful in renal calculi, dysurea, retention of urine and skin disorders with discharges like allergies, urticaria and other erythematous disorders.[3,5]

The fruits of *T. chebula* is rich in tannins (about 32%-34%) and its content varies with geographical distribution.[6,7] The tannins of T. chebula are of pyrogallol (hydrolysable) type. The plant is found to contain gallic acid, chebulagic acid, punicalagin, chebulanin, corilagin, neochebulinic acid, ellagic acid, chebulinic acid, phloroglucimol and pyrogallol, along with phenolic acids such as ferulic, p-coumaric, caffeic and vanillic acids. [8, 9]

It possesses many pharmacological activities such as antioxidant and free radical scavenging activity [10], Hepatoprotective activity [11], Cytoprotective activity.[12] Antidiabetic and renoprotective activity[13,14], Antibacterial activity[15], Antifungal activity[16], Antiviral activity [17, 18]. Antiprotozoal activity [19], Anti-inflammatory and anti-arthritic activity [20], Adaptogenic and antianaphylactic activities[21], Hypolipidemic and hypo-cholesterolemic activity[22, 23]. Gastrointestinal motility improving and anti-ulcerogenic activity [24, 25], Antispasmodic activity [26], Wound healing activity[27] and Immuno-modulatory activity[28]. The purpose of this study was to compare different parts of this plant for its pharmacognostic standardization.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

1,1Diphenyl -2-picrylhydrazyle (DPPH), was obtained from Sigma Aldrich Co. All other chemicals used were of analytical grade.

2.2 Plant material

The plant specimen i.e. leaves, fruits and bark of *T. chebula* were collected from CSIR-NBRI, India in 2016. The plant was authenticated by Dr. AKS Rawat, NBRI. A voucher specimen has been submitted in institute's herbarium.

2.3 Preparation of Plant Extracts

The fresh plant material was collected, thoroughly washed with water to remove all debris and then shade dried. The dried material

was powdered by using electric grinder at 100 mesh size. Extraction was performed by soxhlation process in two steps. Firstly the powdered material was defatted under soxhlet assembly using 250mL of 98% ether for 6 hours. This is followed by 9 hours soxhlation of defatted powder by using 250mL of chloroform as solvent. Same procedure was used to obtain ethanolic extract, after defatting of crude drug it was extracted with 250mL of 99.9% ethanol for 6 hours.

The final extracts were passed through Whatman No. 1 filter paper. The filtrates obtained were concentrated under vacuum in a rotary evaporator at 40 °C and stored at 4 °C for further use. The crude extracts were obtained by dissolving a known amount of dry extract in 98% methanol to obtain a stock solution of 1000 µg/ml. The stock solutions were serially diluted with the respective solvents to obtain lower dilutions (25, 50, 100, 125,150, 200, 250, 300 and 500µg/ml).

2.4 Physicochemical and Phytochemical Studies

Physicochemical and Phytochemical studies like extractive values, total ash, acid insoluble ash, total sugar, starch, tannin, and phenols were calculated from the shade-dried and powdered (60 mesh) plant material [29-32].

2.5 Antioxidant Activity (DPPH Free Radical Scavenging Activity)

Antioxidant activity of the plant extracts and standard was assessed on the basis of the radical scavenging effect of the stable DPPH free radical by the method previously described by 21. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as the standard in solutions ranging from 1 to 50µg/ml. 0.002% DPPH solution in methanol was prepared. Then 2 ml of this solution was mixed with 2 ml of sample solutions (ranging from 25µg/ml to 500 µg/ml) and the standard solution to be tested separately. These solution mixtures were kept in the dark for 30 min and optical density was measured at 517 nm using a Shimadzu spectrophotometer against methanol. The blank used was 2 ml of methanol with 2 ml of DPPH solution (0.002%). The optical density was recorded and percentage of inhibition was calculated using the equation: % of inhibition of DPPH activity = (A–B) /A × 100; where A is optical density of the blank and B is optical density of the sample.

2.6 HPTLC Studies

Air dried (45-55°C) powdered leaves; fruits and bark of *T. chebula* (2.0g) in triplicate were extracted separately with 3X20 ml methanol. Extracts were concentrated under vacuum and re-dissolved in methanol, filtered and finally made up to100 ml with methanol prior to HPTLC analysis. Reagents used were from Merk (Germany) and standard lupeol was procured from Sigma-Aldrich (Steinheim).

2.7 Chromatographic Conditions

Chromatography was performed on Merk HPTLC precoated silica gel 60 GF_{254} (10x10cm) plates. Methanolic solutions of samples and lupeol of known concentrations were applied to the layers as 6 mm-wide bands positioned 15 mm from the bottom and 15 mm from side of the plate, using Camag Linomat V automated TLC applicator with nitrogen flow providing a delivery speed of 150nl/s from application syringe. These conditions were kept constant throughout the analysis of samples.

2.8 Detection and Quantification of Lupeol

Instrumentation Conditions

The following were the instruments and chromatographic conditions used. Spotting device: Linomat V automatic sample applicator; CAMAG (Muttenz, Switzerland), Syringe: 100 µL Hamilton (Bonaduz, Switzerland). TLC chamber: glass twin trough chamber (20 × 10 × 4 cm); CAMAG. Densitometer: TLC Scanner 3 linked to winCATS software V.4.06; CAMAG. HPTLC plates: 10 × 10 cm, 0.2 mm thickness precoated with silica gel 60 F_{254}; E. Merck (Darmstadt, Germany). Experimental conditions: temperature, 25±2°C; relative humidity, 40%. Solvent system: toluene–ethyl acetate–formic acid (8:2:0.1). Detection wavelength: 500. Visualization agent: Anisaldehyde-Sulphuric acid reagent. Slit dimension: 5.00 × 0.45 mm. Scanning speed: 10 mm s^{-1} and source of radiation: deuterium lamp.

Table 1. Phytochemical analysis in methanolic extract of *Terminalia chebula* Fruit, Bark and Leaves

Phyto-constituents	Tests	Observations		
		Fruit	Bark	Leaves
Carbohydrate	Molisch's test	+	-	-
	Fehling's test	+	+	+
	Caramelisation	+	-	+
Tannins	5% $FeCl_3$ test	-	+	+
	5% NaOH test	+	+	+
Alkaloids	Dragendroff's test	+	+	+
	Hager's test	-	+	-
	Wagner's test	-	-	+
Flavonoids	Zn HCl test	+	+	+
Sterols and triterpenoids	Libermann burchard's test	+	-	+
Saponins	Foam test	+	+	+
Proteins and Amino acids	Biuret test	+	+	-
	Ninhydrin test	-	+	-
Reducing sugars	Fehling's test	+	-	-

*Present (+), Absent (-)

3. RESULTS

3.1 Phytochemical screening

Phytochemical screening for the hydro-alcoholic extract showed positive test for flavonoids, proteins, carbohydrates, glycosides, phenolic compounds and saponins etc. Results are shown in Table 1.

3.2 Physicochemical Studies

Parameters such as extractive values (water and alcohol soluble), total ash and acid insoluble ash values, total phenolic content, total tannin content, and total flavonoids were determined. Results are shown in Table 2-6.

Table 2. Determination of Total Ash value and Acid Insoluble Ash value

Observation	Total Ash value			Acid insoluble ash value		
	Bark	Leaf	Fruit	Bark	Leaf	Fruit
Range (%)	9-10	8-9	6-7	6-7	7-8	0.20-0.25
Average (%)	9.14	8.16	6.30	6.44	7.65	0.23

*Range (%) for table 2 were taken from three readings of test sample.

Table 3. Determination of Extractive value

Extracts	Bark	Leaves	Fruit
Alc. Soluble Ext. (%)	9.5-10	7-8	9-11
Average (%)	9.83	7.60	9.66
Water soluble Ext. (%)	19-20	24-25	20-21
Average (%)	19.44	24.36	20.16

*Range (%) for table 3 were taken from three readings of test sample.

Table 4. Total Phenolics in extract of *Terminalia chebula* Fruit, Bark and Leaves

Observation	Fruit	Bark	Leaves
Total phenolic (%)	0.35	1.26	1.97

Table 5. Total tannins in extract of *Terminalia chebula* Fruit, Bark and Leaves

Observation	Fruit	Bark	Leaves
Total tannins (%)	0.219	0.209	0.083

Table 6. Total Flavonoids in extract of *Terminalia chebula* Fruit, Bark and Leaves

Observation	Fruit	Bark	Leaves
Total Flavonoids (%)	0.751	0.74	0.688

3.3 Antioxidant activity

Invitro antioxidant study of leaves; fruits and bark of *T. chebula* was performed using ethanolic extract. In this study fruit extract showed the least IC_{50} value of 0.13 mg/ml followed by bark and leaves. Standard Quercetin showed IC_{50} value of 0.0072 mg/ml. Results are shown in Table 7.

Table 7. Antioxidant Evaluation by DPPH Assay

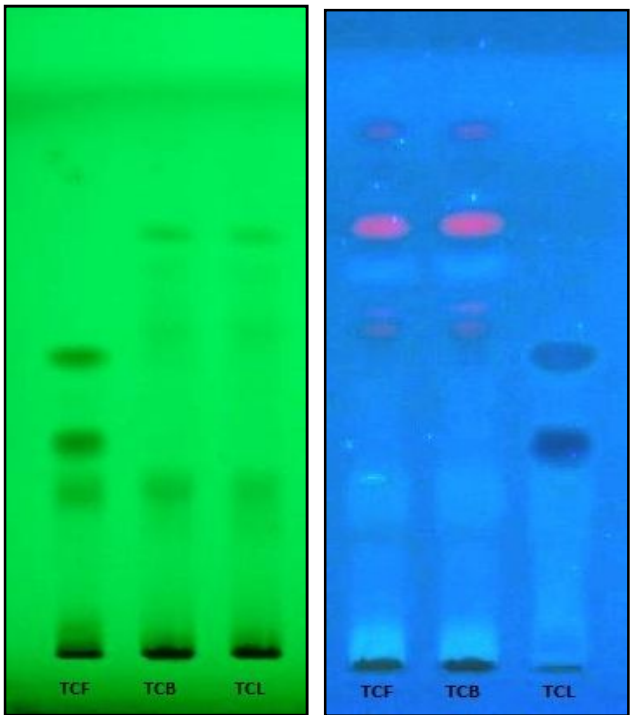
Samples	Conc. (mg/ml)	% Inhibition	IC_{50} (mg/ml)
T. chebula Fruit	0.05	24.94	0.13
	0.1	40.31	
	0.15	57.06	
	0.2	72.77	
T. chebula Bark	0.05	20.5	0.16
	0.1	34.05	
	0.15	47.26	
	0.2	59.56	
T. chebula Leaves	0.125	19.23	0.42
	0.25	38.19	
	0.5	58.9	
	0.75	77.5	
Quercetin	0.0025	16.96	0.0072
	0.005	35.39	
	0.0075	53.93	
	0.01	67.86	

3.4 HPTLC Studies

Quantification of lupeol in the samples of *T. chebula* fruit, bark & leaves has been performed and was found to be 0.042%, 0.024% and 0.034% respectively, Table 8. A Densitogram and Banding pattern obtained from extract shows lupeol as shown in Figure 1 & 2.

Table 8. Quantitative analysis of lupeol in extract of Fruit, Bark and Leaves by HPTLC

Plant material	%Lupeol
Fruit	0.042
Bark	0.024
Leaves	0.034



A B

Fig.1 HPTLC fingerprinting profile of Terminalia chebula at (A) 254nm & (B) 366nm

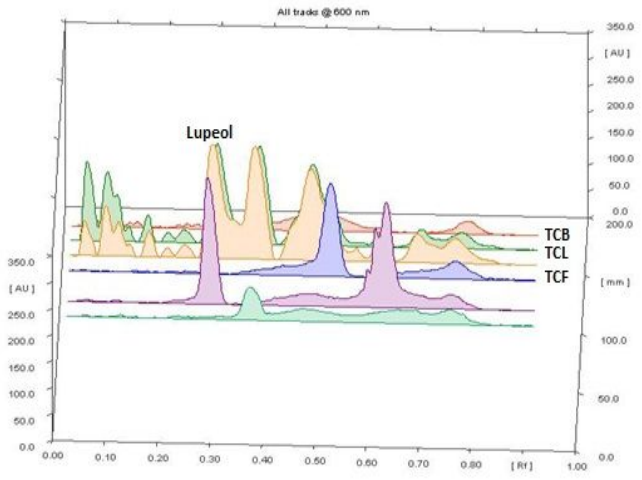


Fig.2 HPTLC Densitogram showing peaks of plant samples and lupeol

4. DISCUSSION

Quercetin (standard compound) showed highest significant and potent antioxidant activity in DPPH free radical scavenging method. Chloroform extract showed the most potent activity followed by ethanolic extract.

Presence of phenolic compounds in *T. chebula* suggests that the antioxidant activity may be due to the polyphenolic content. Identification of all chemical constituents in extract those are responsible for antioxidant activity requires further investigation, the crude extracts merits further experiments *in vivo*. Lupeol present in this species, has been studied through HPTLC. The presence of lupeol has not yet been compared in different parts in this species which may be utilized for the proper standardization of the drug. Thus, the effective source of *T. chebula* could be employed in all medicinal preparations to combat myriad diseases associated with oxidative stress and related disorders.

5. ACKNOWLEDGEMENTS

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6. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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